SALMONELLOSIS IN INDONESIA: PHAGE-TYPE OF <u>SALMONELLA</u> <u>ORANIENBURG</u> OBTAINED FROM HOSPITALIZED PATIENTS IN JAKARTA, INDONESIA

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Salmonellosis in Indonesia: phage-type of *Salmonella oranienburg* obtained from hospitalized patients in Jakarta, Indonesia

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SUMMARY

During a survey in Jakarta, Indonesia, 158 cultures of *Salmonella oranienburg*, consisting of two phage types, were obtained from 150 hospitalized patients with diarrhoea. Phage type I, though found notably in young children, was found in all age groups while phage type II was found almost exclusively in young children aged 0–7 years. Phage type I may produce a more severe clinical picture affecting all age groups alike, while phage type II may result in hospitalization of only the very young, who are more susceptible to dehydration. Phage type I was significantly more resistant than phage type II to the individual antibiotics: tetracycline, chloramphenicol, kanamycin and neomycin. However, there was no difference in their respective antibiotic resistance patterns as measured by disk and MIC assay. All cultures were sensitive to gentamicin and trimethoprim-sulphamethoxazole 1:19.

INTRODUCTION

Salmonellosis is a worldwide public health problem. Although *Salmonella typhimurium* is the most common serovar throughout the world (Anderson *et al.* 1978), *S. oranienburg* is the most frequently isolated *Salmonella* from hospitalized patients in Jakarta, Indonesia.

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Considerable information is available on the epidemiology, microbiology and clinical features of salmonellosis (Rubin & Weinstein, 1977). However, the correlation of the phage-types of *Salmonella* serovars with clinical data is limited despite its importance to physicians and epidemiologists. In this paper we report the finding of a difference in age response of the host population to two phage-types of *S. oranienburg* obtained from hospitalized patients in Jakarta, during the period February 1977 to March 1978.

MATERIALS AND METHODS

Patient group

Clinical specimens were received from patients suffering from diarrhoea on 4 adult medical wards and 3 paediatric wards from hospitals in Jakarta. The patients were distributed among all age groups, and from upper-middle to lower socioeconomic levels.

Rectal specimens were collected from all patients whereas cerebrospinal fluid, blood and urine specimens were collected only when indicated.

Isolation and identification

Rectal specimens were collected on Dacron swabs, transported to the laboratory in Amies transport medium (Difco, Detroit, Mich.), enriched with mannitol selenite broth (Arnold, 1956) and plated on SS agar (Difco) and MacConkey agar (Difco), both directly and after enrichment. Blood specimens were collected aseptically; 3 ml were placed in a vial containing 12 ml of 10% ox bile, incubated up to 4 days and subsequently streaked on MacConkey agar. Sediment from centrifuged urine specimens was placed directly on MacConkey and SS agars. Cerebrospinal fluid (CSF) specimens were collected aseptically, plated on 5% defibrinated sheep blood agar, chocolate agar with 1% IsoVitaleX (BBL), Trypticase Soy Agar (BBL) and MacConkey agar. A portion was also inoculated into a 2-phase medium consisting of an agar phase (Columbia agar base [BBL], 42·5 g; dextrose, 2·5 g; L-cysteine HCl, 0·1 g; Na_2CO_3, 0·6 g; 0·1% hemin solution, 0·1 ml; H_2O, 1000 ml) and a broth phase composed of Columbia broth (BBL) with 1% IsoVitaleX (BBL). The blood and chocolate agar plates were incubated in a candle jar. All plates were incubated at 36–37 °C.

Biochemical identification and serotyping were performed by the methods outlined by Edwards & Ewing (1962). Antibiotic sensitivity patterns were determined by the Barry modification of the Kirby-Bauer method (Barry, Garcia & Thrupp, 1970), using Mueller-Hinton Agar (Difco) and the following sensitivity disks (Difco): ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), trimethoprim-sulphamethoxazole 1:19 (25 μg), streptomycin (10 μg), sulphathiazole (250 μg) and tetracycline (30 μg). The minimum inhibitory concentration (MIC) of antibiotics was determined by the agar dilution method (Gavan, Cheatle & McFadden, 1971) using Mueller-Hinton agar, and the following standard reference powders: ampicillin trihydrate, tetracycline HCl and kanamycin sulphate (Bristol Laboratories, Syracuse, N.Y.);

Table 1. *Phage type distribution of* S. oranienburg *in clinical specimens collected from February* 1977 *to March* 1978 *in Jakarta*

Specimen type	Total isolates	Phage type I*	Phage type II†
Rectal	150	124	26
CSF	5	5	0
Blood	2	2	0
Urine	1	1	0

* Phage type I pattern: 1/10/17/18/21/24/25/29/35/36/38/45/50.
† Phage type II pattern: 1/21/29/36/38/48.

chloramphenicol (Parke Davis Co., Detroit, Mich.); gentamicin sulphate (Schering Corp., Kenilworth, N.J.), and amoxycillin trihydrate (Beecham Research Laboratories, Bristol, Tenn.). The MIC was the lowest antibiotic concentration which resulted in no bacterial growth on the medium.

R factor transfer

Transfer of tetracycline resistance from *S. oranienburg* to *Escherichia coli* ATCC 19215 was determined by placing 24 h cultures of *E. coli*, 2·0 ml, and *S. oranienburg*, 0·5 ml, into 10 ml of fresh BHI (Difco) and incubating for 18 h at 36 °C. Tenfold dilutions were plated on MacConkey agar containing 10 μg/ml tetracycline.

Phage typing

The phage typing of *S. oranienburg* was conducted using a set of 50 phages isolated from sewage (Gershman, 1977).

RESULTS

The 158 *S. oranienburg* cultures isolated during the period February 1977 to March 1978 represented two major phage types, of which 132 isolates had the phage pattern 1/10/17/18/21/24/25/29/35/36/38/45/50 while 26 isolates displayed a phage pattern 1/21/29/36/38/48. These two patterns are distinct and different and are referred to in this paper as phage type I and II respectively.

In three cases paired isolates were obtained from a urine and rectal specimen, a blood and rectal specimen, a CSF and rectal specimen; in three cases two successive rectal specimens; and in one case three successive rectal specimens. The phage types of these isolates were consistent with each other, all being phage type I. All phage type II isolates were from rectal specimens and were not found in CSF, blood or urine (Table 1).

Of patients with *S. oranienburg* phage type I, 77 % were less than 6 years of age, with a range of less than 1 month to 57 years; 96 % of patients from whom phage type II cultures were obtained were less than 8 years old, with only 1 patient above 7 years (Table 2). Phage type II appears to be principally a pathogen

Table 2. *Age distribution of patients infected with* S. oranienburg *isolated in Jakarta during February* 1977 *to March* 1978

Age (years)	Phage type I	Phage type II
0–5	96	24
6–7	0	1
8–10	3	0
11–20	6	0
21–30	8	1
31–40	1	0
41–57	3	0
Unspecified	7	0
Total	124	26

of young children, aged 7 or less ($P = 0{\cdot}055$, two-tail, difference of two proportions).

There was no significant difference due to sex of the patients; Type I was found in 62 males and 60 females (2 unspecified) and Type II in 13 individuals of each sex.

In Jakarta there are generally two main diarrhoea seasons during the year, as indicated by hospital admissions. One occurs in the summer during the dry season, and the second in winter during the wet season (Fig. 1*a*). Both peaks of hospital admissions are associated with periods of heavy rain, even in the dry season which usually occurs from April to October. However, the peaks for the isolation of *S. oranienburg* do not coincide with those for hospital admissions (Fig. 1*b*), indicating that the peak diarrhoea season is due to enteropathogens other than *S. oranienburg*. The peaks for *S. oranienburg* followed the hospital admission peak after 1–2 months. *S. oranienburg*, phage type II, as a proportion of all *S. oranienburg* isolated, increased from 3 % to 18 % during the study period (Fig. 1*c*) and is replacing phage type I, which decreased from 96 % to 56 % in the 0- to 7-year-old age group (Fig. 1*d*, $P = 0{\cdot}027$, difference of two proportions).

No differences were noted in signs, symptoms or degree of illness in patients infected with the two phage types of *S. oranienburg* when the medical records were reviewed retrospectively.

Mixed infections were found in 12 patients. *S. oranienburg* phage type I was associated with 5 other enteropathogens in 10 cases, 8 of which were *Vibrio* species, while 2 infections with phage type II were associated with *Vibrio cholera* (Table 3).

Antibiotic-resistant patterns for all isolates of both phage types are given in Table 4. Most of the *S. oranienburg* isolates were multiply resistant to the antibiotics tested, 93 % resistant to 5 or more, with 84·2 % resistant to 7. All isolates were sensitive to gentamicin and trimethoprim-sulphamethoxazole 1:19. There were no significant differences in the patterns of the two phage types. In cases where isolates were obtained from paired specimens, the antibiotic resistance patterns were identical. However, phage type I had significantly greater resistance

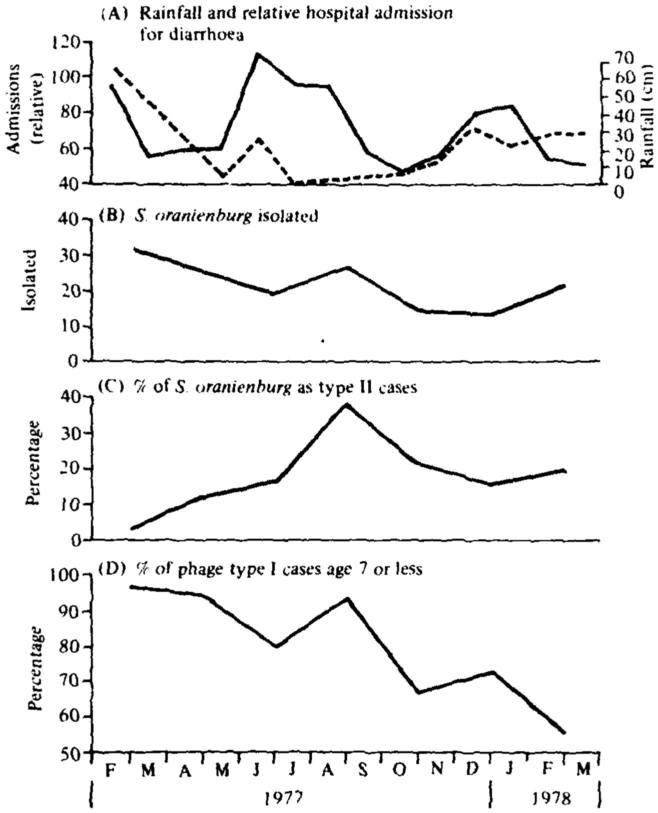

Figure 1. (A) Solid line, relative monthly hospital admissions for diarrhoea in Jakarta, Indonesia; dashed line, rainfall. (B) Actual number of isolates of *S. oranienburg* from hospitalized patients for diarrhoea, bi-monthly. (C) Phage type II as a percentage of total *S. oranienburg* cases, bi-monthly. (D) Phage type I as a percentage of total *S. oranienburg* cases less than 8 years of age, bi-monthly.

Table 3. *Mixed infections occurring with* S. oranienburg

	S. oranienburg	
	Phage type I	Phage type II
Vibrio cholera (Ogawa)	3	2
Vibrio NAG I	3	0
Vibrio parahaemolyticus	2	0
S. agona	1	0
S. typhi	1	0
	10	2

Table 4. *Antibiotic resistance patterns of* S. oranienburg *determined by disk assay*

Antibiotic*	Phage type I	Phage type II
Sensitive to all	0	2
Am	0	1
T	5	0
Am, T	1	1
Tu, Su, S	1	0
Am, C, T, Su, S	1	2
Am, C, T, K, N	4	0
Am, Su, S, K, N	2	1
Am, C, Su, S, K, N	0	4
Am, C, T, Su, S, K, N	118	15
	132	26

* Am, ampicillin; T, tetracycline; Su, sulphathiazole; S, streptomycin; C, chloramphenicol; K, kanamycin; N, neomycin.

Table 5. *Antibiotic resistance by phage type of* S. oranienburg *to individual antibiotics*

Antibiotic	Phage type I	Phage type II	*P* value
Ampicillin	126	24	NS*
Tetracycline	130	18	<0·001
Streptomycin	122	22	NS
Chloramphenicol	123	21	0·044
Kanamycin	124	20	0·005
Neomycin	124	20	0·005
Sulphathiazole	122	22	NS
Gentamicin	0	0	NS
SxT†	0	0	NS
Total cultures tested	132	26	

* NS = Not significant.
† Trimethroprim-Sulphamethoxazole 1:19.

than phage type II for the individual antibiotics tetracycline, chloramphenicol, kanamycin and neomycin (Table 5, difference of two proportions) and greater overall resistance (all antibiotics combined $P = 0{\cdot}001$, even when tetracycline is excluded; $P = 0{\cdot}022$ (Chi squared)).

The MIC values of the isolates, by phage type, to selected antibiotics is presented in Table 6. There were no significant differences in the MIC values of the two phage types to the antibiotics tested with the exception of tetracycline and kanamycin. Phage type I had greater resistance to tetracycline and kanamycin than did phage type II (difference of two proportions), $P < 0{\cdot}001$ and $P = 0{\cdot}045$ respectively. Though the differences in the two phage types are significant, there was still a high level of resistance in phage type II, with 73% of the isolates resistant to tetracycline and 69% resistant to kanamycin. There were no differences in the MIC patterns of the two phage types.

Table 6. *In vitro susceptibility of* S. oranienburg *to selected antibiotics. Distribution of minimal inhibitory concentrations (MIC) by phage type*

Antibiotic	Phage type	MIC (μg/ml) 0·18	0·33	0·56	1·0	1·8	3·3	5·6	10	18	33	≥56
Ampicillin	I	—	—	—	—	13	—	—	—	—	—	119
	II	—	—	—	—	3	—	—	—	—	—	23
Chloramphenicol	I	—	—	—	—	—	1	13	—	—	—	118
	II	—	—	—	—	—	1	4	—	—	—	21
Tetracycline	I	—	—	—	—	—	—	1	—	—	—	131
	II	—	—	—	—	—	1	1	5	—	—	19
Gentamicin	I	49	73	10	—	—	—	—	—	—	—	—
	II	13	13	—	—	—	—	—	—	—	—	—
Kanamycin	I	—	—	—	—	14	3	2	—	—	—	113
	II	—	—	—	—	7	1	—	—	—	—	18
Amoxicillin	I	—	—	—	1	3	8	1	—	—	—	119
	II	—	—	—	1	1	—	—	1	—	—	23

Table 7. *Transfer of antibiotic resistance of* S. oranienburg *isolates to* E. coli *ATCC* 19215

Antibiotic	Phage type	Transferred	Not transferred
Tetracycline	I	112	21
	II	16	3

Though the two phage types differed in their resistance to tetracycline, as measured by disk and MIC assay, there was no difference in their ability to transfer the resistance to a recipient *E. coli* by conjugation (Table 7).

DISCUSSION

S. oranienburg appears to be the most frequently isolated serovar from hospitalized diarrhoea patients in Jakarta. During the 14 months of this study, isolation of phage type II increased from 3% to 18%, though the isolation of *S. oranienburg* was in a declining trend during this same period. This increase in phage type II may reflect some portion of a cyclic pattern that has existed for some time or may be due to its recent introduction and establishment as a new phage type to this host population.

The host population, in this study, can be divided into four groups; phage type I children and adults and phage type II children and adults. *S. oranienburg* was isolated from hospitalized patients in three of the four groups, and was able to invade the host, cause acute enteritis and produce fluid loss (Giannella *et al.* 1973). One explanation for the lack of representation of phage type II adult infections in these hospitalized patients may be due to a difference in virulence of the two phage types. The findings of a single isolate in an adult occurred in August 1977, during the period of peak isolation of phage type II, and will be ignored for this discussion.

Many of the determinants of pathogenicity are cell-surface components of the bacteria (Smith, 1972) and the lack of one of the members of these required determinants may result in the loss or attenuation of virulence (Smith, 1958). The *O*-antigen of the lipopolysaccharide (LPS) is important in the virulence of Salmonella (Makela, Valtonen & Valtonen, 1973). Slight alterations in the *O*-side chains of *S. typhimurium* LPS result in changes in the virulence of the organism for mice (Makela *et al.* 1973; Valtonen, 1969). Also, phage adsorption is dependent on the structure of these *O*-side chains, with the phage able to discriminate slight chemical changes (Lindberg, Sarvas & Makela, 1970). It can then be expected that the same changes to LPS that are involved in determining phage type may also be associated with virulence of the organism. Saragea, Maximescu & Meiterb (1973) demonstrated that the clinical form of diphtheria was related to the phage type: *Corynebacterium diphtheriae* phage type XIV produced a milder clinical evolution of the disease than type IX.

Bacterial plasmids can carry information which may result in alterations in the LPS of the outer membrane (Derylo *et al.* 1975) or result in phage resistance (Yoshikawa & Akiba, 1962) and other characteristics (Novick, 1969) in addition to coding for antibiotic resistance. The correlation between the resistance to several antibiotics and virulence of a pathogenic organism is complicated and the results are not consistent (Cutler, 1979; Krynski, Kedzia & Kaminska, 1964; Lacey & Chopra, 1975; Namavar *et al.* 1978).

The basis of the difference in virulence probably is related to alterations in LPS associated with the determinants for phage type, with possible contributions from plasmid-mediated factors. However, the relative susceptibility or resistance of the host remains important, since it has been suggested that the main role of LPS in bacterial virulence is to provide protection for the bacteria against host defence mechanisms (Valtonen, Sarvas & Makela, 1971).

A slight difference in virulence determinants of the pathogen and the interplay with the host defence mechanisms, associated with age, probably results in two degrees of severity of diarrhoea produced by *S. oranienburg*. Phage type I strains may produce a more severe clinical picture, in sufficient intensity to result in hospitalization of both the children and adult groups alike. Phage type II, on the other hand, may produce a milder disease, with less severe symptoms or for a shorter duration. This would result in the hospitalization of only the very young, who are more susceptible to dehydration, with adult patients without complications being treated at home or in the clinic, and consequently not seen in this study.

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